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In addition to intensively managed ex-situ populations, DNA profiling methods can be applied to remaining in-situ populations in order to understand a variety of ecological parameters such as population structure (i.e. the number of populations in the wild and how isolated they are from one another), relative genetic variability, individual dispersal, census size and breeding behavior. This approach is particularly useful for studying rare or inconspicuous species such as the pygmy hippo, where actual animal sightings are uncommon and DNA samples can be obtained from collection of faecal material.

DNA profiling is based on the measurement of multiple points, or DNA markers, within a species genome. These DNA markers usually vary among species, requiring new sets of markers to be developed for every new species under study. Researchers from around the world are currently collaborating to develop sets of pygmy hippopotamus DNA markers to support both ex-situ and in-situ conservation management. The work has relied upon the contribution of EEP holders to provide high quality blood samples for the initial phase marker development. The technique being used, known as RAD-sequencing, uses state-of-the-art high throughput DNA sequencing techniques similar to those used to sequence the human genome. With RAD sequencing, sequences from different control samples are compared at hundreds of thousands of regions in the pygmy hippo genome to discover areas that show variability, or 'polymorphism'. The polymorphisms are known as SNPs and are the latest kind of DNA marker used in conservation genetics.

To date, samples have been processed to extract DNA and create the RAD libraries that contain all of the DNA regions under analysis. These libraries have subsequently been sequenced, resulting in the production of billions of base pairs (letters) of DNA sequence data. At the present time the data is being subject to quality control analysis prior to processing for the discovery of SNP DNA markers. It is hoped that the markers will be available for use by late spring of this year. The work is being carried out by the Wildgenes laboratory at the Royal Zoological Society of Scotland in partnership with Chester University, the University of Edinburgh and the European pygmy hippo EEP.

5.4 Weight loss in pygmy hippos (*Choeropsis liberiensis*)

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Introduction

Obesity in captive animals is frequently reported in zoo literature. The problem is generally attributed to the relative oversupply of highly digestible feeds, such as commercial fruits and cereal-based pellets, and overfeeding (Clauss *et al.* 2009). In the wild, pygmy hippos (*Choeropsis liberiensis*) feed on ferns, herbs, wild fruits and little or no grass (Hentschel, 1990; Eltringham, 1999). No wild body weights (BW) have been reported, but camera trap photos of wild individuals are available (Collen *et al.* 2011). Captive body weights range from 160–260kg (Lang, 1962; Crandall, 1964; Walker, 1968; Schwarm *et al.* 2006), with many pygmy hippos appearing considerably larger in comparison to images of their wild conspecifics (pers. obs.). Pygmy hippos are non-ruminating foregut fermenters, with particularly long digesta retention times (Clauss *et al.* 2004; Schwarm *et al.* 2008). In addition, Schwarm *et al.* (2006) found that the four pygmy hippos in her study had a daily digestible energy intake (DEI) of 0.30 ± 0.11 MJ/kg metabolic body weight ($\text{kg}^{0.75}$), which is substantially lower than most other ungulates that require 0.49–0.66 MJ/kg^{0.75}/d. Thus, pygmy hippos may have low metabolic rates, with estimated energy requirements of only 45–61% of the calculated interspecies mean, which could explain their susceptibility to obesity when fed energy-dense diets in captivity. Lintzenich and Ward (1997) recommended 30% pellets to 70% roughage, with 14.4–20% crude protein (CP; recalculated on a % dry matter [DM] basis) and 34–41% neutral detergent fibre (NDF as % DM). The aim of this study was to reduce the body weight (BW) of two pygmy hippos at Bristol Zoo Gardens and investigate the feeding recommendations for pygmy hippos.

Methods

The feeding trials were performed with one male (Nato) and one female (Lise) pygmy hippo at Bristol Zoo Gardens, UK. The animals were 27 and 24 years old, respectively, at the start of the trial in 2007 and were both born and parent-reared in captivity. Both pygmy hippos were trained to walk onto scales to monitor BW. At the start of the trial Nato weighed 278kg and Lise weighed 287kg, and the aim was to reduce BW to a target range of 230–250kg. Both animals were continually monitored under veterinary supervision throughout the feeding trials. The weight loss occurred in two stages. The original diet, consisting of approximately 2.5kg of cereals and pellets, half a loaf of bread, half a bucket of fruit and vegetables and lucerne hay per animal per day, was gradually replaced over a period of one month by a weight-loss diet (Diet 1) consisting of *ad libitum* lucerne hay, with 550g of MazuriTM Browser Maintenance pellets (MazuriTM Zoo Foods, Essex, UK) and 800g of a mixture of iceberg lettuce and baby leaf spinach per animal. After the diet transition, the pygmy hippos were continued on Diet 1 for nine-and-a-half months between 15th March 2007 and 8th February 2008. Once both pygmy hippos were within the target BW range of 230–250kg, both pygmy hippos were then placed on Diet 2a, a maintenance/less-intense weight-loss diet of *ad libitum* lucerne hay with 1kg of pellets and 1kg of green vegetables per day. While the animals were on Diet 1, and during the first five weeks on Diet 2a, food intake was measured by weighing individual food items offered and subtracting the weight of the food not consumed on a daily basis. Feed samples were taken and analysed for their dry matter (DM), crude protein (CP), lipid and neutral detergent fibre (NDF) content. The intake of roughage, pellets and overall nutrient intake was compared to the feeding recommendations of

Lintzenich and Ward (1997). After one year on Diet 2a, the browser maintenance pellets were replaced by Emerald Green Feeds Grass Pellets (A. Poucher and Sons, Market Rasen, UK) for the remaining pygmy hippo on 27th March 2009 (Diet 2b) (the female was euthanized on 7th November 2008 for an unrelated medical condition). Pygmy hippos were weighed twice weekly during Diet 1 and weekly thereafter, when possible, until January 2012.

Results

On Diet 1, lucerne hay comprised 72–75% of DM intake, with a slight decrease to 65–67% on Diet 2a. Lucerne hay intake increased on Diet 2a, despite *ad libitum* availability on both diets (Table 1). The lucerne hay used comprised of 10.8–12.2% CP and 58.3–61.7% NDF. The roughage-to-pellet ratios were comparable to the recommendations of Lintzenich and Ward (1997) of 30% pellets to 70% roughage. The intake of vegetables on both diets was minimal, contributing just 1.9–3.5% of the DM content of the diet. The CP content of the diet was lower than the recommendations of Lintzenich and Ward (1997) (14.4–20% DM), at 11.6% on Diet 1 and 12.6–12.7% on Diet 2a. Conversely, both diets contained approximately 52% NDF, which is higher than the recommendations of 34–41% NDF. While on Diet 1, Nato's BW decreased from 278kg to 244kg, a loss of 34kg or 12.3% BW, and Lise's BW decreased from 287kg to 245kg, a loss of 42kg or 14.6% BW (Fig. 1). The BW of Nato, the remaining male pygmy hippo, continued to decrease gradually for eight months on Diet 2a, then fluctuated between 230–240kg on Diet 2b (Fig. 2). No abnormal behaviours were observed during the feeding trials. Faecal consistency improved on the grass pellets.

Table 1. Dry matter (DM) and nutrient intake by Nato and Lise at Bristol Zoo Gardens on Diet 1 and Diet 2a.

	Diet 1		Diet 2a	
	Nato	Lise	Nato	Lise
Mean lucerne intake (g DM/d)	1393	1608	2158	2472
Mean pellet intake (g DM/d)	492	500	890	1018
Mean vegetable intake (g DM/d)	40	40	89	134
Total intake (g DM/d)	1925	2148	3137	3622
Crude protein (CP; g/d and as a % of DM)	223 (11.6%)	248 (11.6%)	397 (12.6%)	462 (12.7%)
Lipids (g/d and as a % of DM)	37 (1.9%)	41 (1.9%)	57 (1.8%)	68 (1.9%)
Neutral detergent fibre (NDF; g/d and as a % of DM)	994 (51.7%)	1116 (51.9%)	1663 (53.0%)	1870 (51.6%)



Figure 1. Comparison of the body condition of (a) pygmy hippo before the start of the trial and (b) pygmy hippo towards the end of Diet 1.

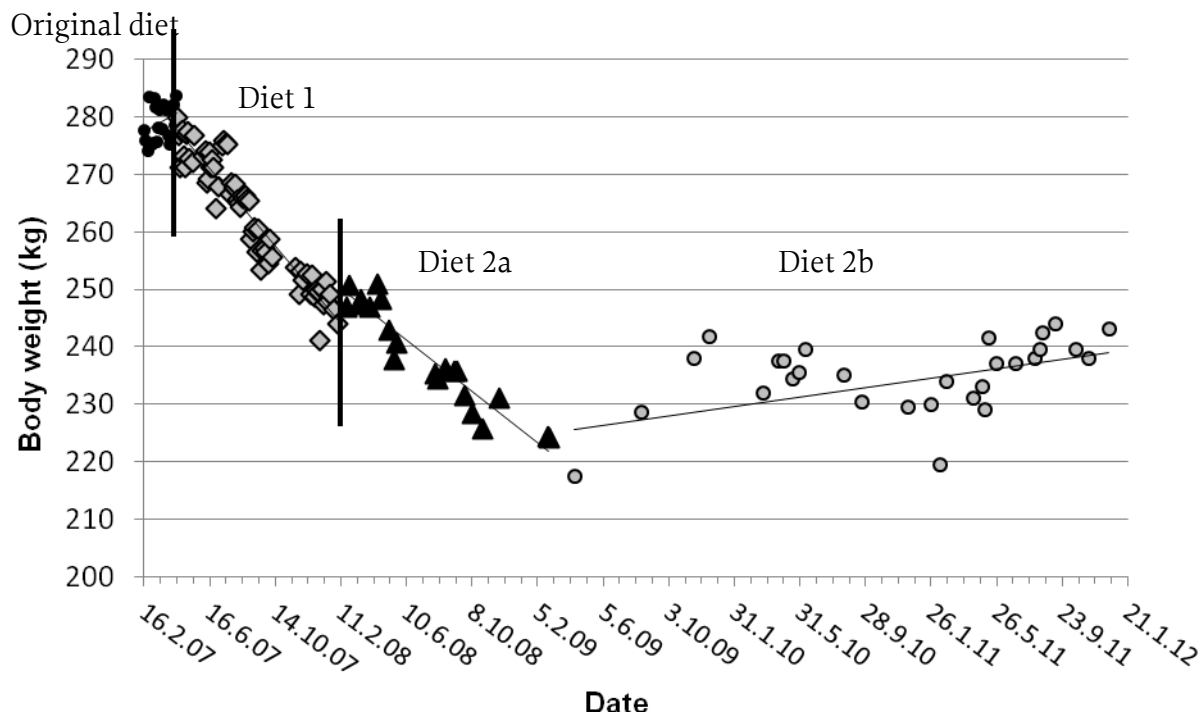


Figure 2. Body weights of male pygmy hippo Nato at Bristol Zoo Gardens on the original diet, Diet 1, Diet 2a and Diet 2b.

Discussion

The results of this study showed that reducing the content of highly digestible feeds in the diet can lead to weight loss in pygmy hippos. The most substantial dietary change was reducing the concentrate feed from 2.5kg pellets and cereals per animal and day to 0.55kg pellets on Diet 1 and 1.0kg pellets on Diets 2a and 2b. On both Diet 1 and Diet 2a, the limited quantities of pellets and *ad libitum* access to lucerne hay resulted in a proportion of roughage to pellets that was similar to the recommendations of Lintzenich and Ward (1997) of 70% roughage. For training purposes, the green vegetable content of the diet was increased on Diets 2a and 2b, but, due to their high water content, vegetables did not contribute substantially to the overall dry matter intake (1.9–3.5%). However, it should be noted that training was only conducted using green vegetables, as fruits are not recommended due to their high sugar content (Schwitzer *et al.*, 2009). The CP content of

both Diet 1 (11.6% DM) and Diet 2a (12.6–12.7% DM) was substantially lower than the recommendations of Lintzenich and Ward (1997; 14.4–20% DM). Reasons for these high recommendations are unclear, and as in other herbivores, lower dietary protein contents should be acceptable for animals with maintenance requirements only. For example, the current feeding recommendations for captive giraffes (*Giraffa camelopardalis*) have also recommended reducing the CP content for animals with maintenance requirements to 12% (average of Schmidt and Barbiers, 2005) to 14% (Hummel and Clauss, 2006), which suggests the CP recommendations of Lintzenich and Ward (1997) are too high. Both pygmy hippos successfully lost weight during this trial by increasing the proportion of roughage and NDF and decreasing the content of highly digestible feeds in their diet. Nato's weight loss continued through Diets 1 and 2a, but finally remained between 230-240 kg on Diet 2b. Rather than indicating a difference in the nutritional value of the two different pelleted products, this probably indicates a long-term adjustment of the animal to the new feeding situation and to a regular maintenance BW. The results of this study demonstrate that reducing BW is best approached by continuous monitoring of the animals' condition.

Conclusion

Pygmy hippos successfully lost weight and then maintained a lower body weight on a forage-based diet with a limited quantity of pellets.

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